Fermentation of Nypa Sap to Bioethanol

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Abstract*— Indonesia is targeted to produce bioethanol in 2025 about 9,1 million kilo liters [1]. One of the potential materials to be processed to bioethanol is nypa sap since it has a high content of sugar. Moreover, the availability of nypa plantation areas in Indonesia about 973,205.54 ha and the high sugar content make nypa sap is a potential for fermentation to bioethanol. Bioethanol is alcohol which is obtained from biomass fermentation process that is aided by microorganism. The microorganisms of fermentation are Saccaromyces cerevisiae and Pichia stipitis. The optimum condition of bioethanol production from nypa sap was determined by volume of subsrate, volume of inoculum, and the fermentation time. Acquisition optimum concentration of bioethanol by using microbial Saccaromyses cerevisiae is 14% (v/v) and 97.969% with a yield of 10% volume starter and fermentation time 36 hours. The optimum results generated for microbial fermentation using Pichia stipitis is lower at 9% (v/v) ethanol concentration and yield of 92.244% with 15% volume starter and fermentation time 72 hours. These results indicate that the ability of Saccaromyces cerevisiae better than Pichia stipitis in converting sugar to bioethanol.

Keywords—bioetanol; fermentation; nypa sap; Pitcia stipitis; Saccaromyces Cerevisiae

I. INTRODUCTION

The availability of fossil fuels is expected to be exhausted in the next 20 years. Therefore, introducing of new alternative feedstock to produce renewable energy is needed. One of the efforts that have been made by Directorate of new renewable energy and energy conservation Indonesia is supporting the provision of long-term national energy such as bioethanol. Directorate of new renewable energy and energy conservation has targeted the production of bioethanol Indonesia to 9.1 million kilo liters in 2025 [1]. To achieve this target, it is needed a variety of raw material for bioethanol production.

Nypa is one of the mangrove plants that its sap can be used to be a feedstock for bioethanol production. Nypa sap is potential to produce 15.600 liters of ethanol per ha or two times the results obtained from sugar cane, and 6 times from corn [2]. Nypa is one of the main species in mangrove area with the composition about 30% of the total mangrove area [3]. According to observation data of Center Survey of Natural Resources Marine, the total area of mangrove in Indonesia is 3,244,018.46 ha [4] which about 973,205.54 ha is nypa forest.

In Indonesia, Nypa forest area can be found in Sumatera, Kalimantan, Sulawesi, Maluku, and Irian Jaya. Riau Province is one of the largest nypa forests in Sumatera There are about 41,530.09 ha nypa forest that stretches along the coast of Rokan Hilir and Indragiri Hilir (districts in Riau Province) [5]. The potential of nypa has not been fully utilized. Nypa sap had only consumed as a beverage. While, the leaves are traditionally used as material for roofing, walls, wicker baskets and various cigarette wrappers (wrapping sheets for rolling tobacco). Besides nypa has other benefits which is to be raw material for bioethanol production.

Nypa sap is obtained by tapping the the flower stalks of nypa that have not bloomed. One nypa stalk could produce about 3 liters of sap per day which approximately contain 15-17% sugar brix [6]. Each clump of nypa usually has 4 fruit stalks at the same time that could be harvested about 20 days continuously. Thus, one nypa could produce 12 liters of sap per day [7].

Fermentation is one way of producing bioethanol from nypa sap. Fermentation is a process of the chemical changes of an organic substrate by biochemistry catalyst action, which is enzyme that is produced by certain microbes. During fermentation, feedstock is converted to alcohol. Sucrose content of feedstock is broken down to glucose, and glucose fermentation produces alcohol [8]. Basically, fermented alcohol derived from glucose conversion. *Saccharomyces cerevisiae* is usually used as supporting microbes for fermentation. In this study, bioethanol production from nypa sap fermentation using *Saccharomycess cerevisiae* would be compared with using *Pichia stipitis*. Bioethanol is produced from the fermentation product nypa sap. Bioethanol is alcohol which is obtained from biomass fermentation process that is aided by microorganism. One of the uses of bioethanol is a renewable energy source that can replace fossil fuel as well as gasoline fuel mixtures.

II. MATERIAL AND METHODS

A. *Material*

The alcohol fermentation was conducted in laboratory scale. The sap as feedstock was collected from Nypa forest in Riau Province,

B. *Microbes*

Saccaromyces cerevisiae and *Pichia stipitis* were used as microbial culture in this work. *Saccaromyces cerevisiae* and

pichia stipitis were grown in the nypa sap was applied as starter for fermentation. In order to support the microbial growth, the NPK solution was added as nutrition.

C. *Fermentation conditions and analyses*

The medium fermentation was prepared from 8 L and 50 L nypa sap, 0.4 g/L of urea contained 46% N, and 0.5 g/L of NPK contained 16% P. The medium fermentation was sterilized using autoclave at temperature 121^{o}C for 15 minutes then cooled to room temperature. The initial glucose concentration of medium fermentation was analyzed using visible spectrophotemeter. The experiments were caried out in a 70 L biofermentor with mixer (200 rpm) containing 10% volume of inoculums at anaerob condition and room temperature (25-30^{o}C). The samples were taken for certain time of fermentation, i.e. 24, 36, 48, and 72 hours. The fermentation product then was distilled to separate it from impurities. The concentration of bioethanol was determined using alcohometer, while the glucose concentration by Nelson-Samogyi method.

III. Results And Discussions

A. *The fermentation of nypa sap to bioethanol by using Saccaromyces cerevisiae*

The optimum condition is determined by variations in the volume of substrate, volume of starter, and fermentation time. Volumes of substrates are used 8L and 50L.

In figure 2 provides profile of optimum condition for fermentation nypa sap to bioethanol at 8L of substrate volume is at 10% with a variation starter concentration of bioethanol produced as much as 12% (v/v) for 48 hours. While the concentration of ethanol produced in order conditions i.e. 15% volume starter and 20% volume starter is 10% (v/v) in the span of hours to- 12 to 72 hours.

The differences in concentration of ethanol produced is influenced by the number of volumes of starter are used. After the optimum fermentation conditions is reached, along with increasing volume of starter then causing the number of substrates used for the greater development of microorganisms, so the number of products produced less and less [9].

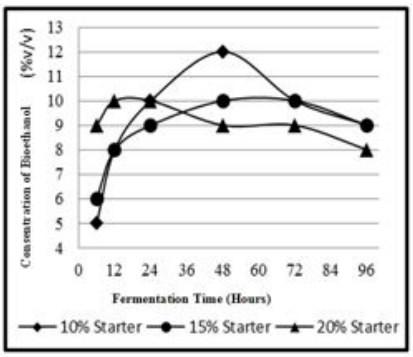

Fig 1. The bioethanol consentration of fermentation nypa sap results using *Saccaromyces cerevisiae* with substrate volume 8L

However, the condition of the substrate volume of 50L with 10% starter volume produced the highest ethanol concentration of 14% (v/v) for 36 hours. Whereas, the volume of 15% starter also produced ethanol concentration of 14% (v/v) for 36 hours. Volume of 20% starter on the same substrate conditions produce bioethanol by 8% (v/v) for 24 hours. The following is a profile of optimum fermentation conditions using substrate volume 50L.

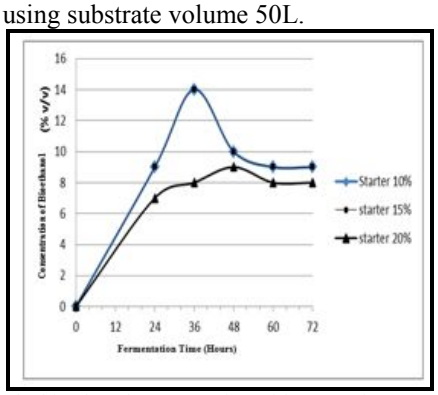

Fig 2. The bioethanol consentration of fermentation nypa sap results using *Saccaromyces cerevisiae* with substrate volume 50L

B. *The fermentation of nypa sap to bioethanol by using Pichia stipitis*

The optimum conditions were obtained at fermentation of nypa sap that uses Pichia stipitis is shown in figure 3, which is the volume of starter 15% bioetanol obtained with a concentration of 9% (v/v) for 72 hours.

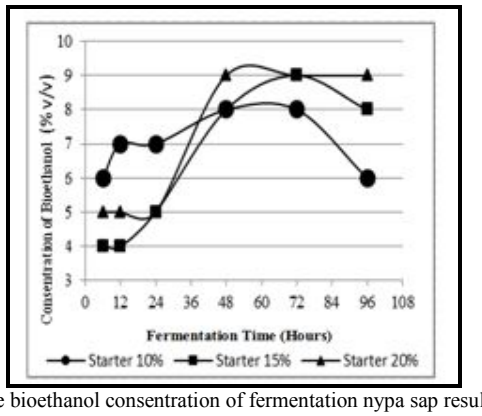

Fig 3. The bioethanol consentration of fermentation nypa sap results using *Pichia stipitis*

In figure 3 provides information that starter volume 15% and 20% resulted in the highest ethanol concentration of 9% (v/v). However, the volume of 15% starter, fermentation time to achieve the optimum conditions required for longer than the 20% starter volume. This is caused by the amount of starter volume 15% less, so that the time reqiured to convert substrates to products more slowly.

C. *The comparison of bioethanol production by fermentation of nypa sap on different variations*

Production of bioethanol by fermentation nypa sap produce different concentrations. Comparison of the concentration of ethanol produced can be seen in Table 1.

TABLE 1. THE COMPARISON OF BIOETHANOL PRODUCTION BY FERMENTATION OF NYPA SAP ON DIFFERENT VARIATIONS.

Volume of Fermentation	Volume of Starter	Consentration of Bioethanol		Yield of Bioetanol	Microorganism
L	%	%	mg/ml	%	
50 [11]	10	14	110,502	97,969	*Saccharomyces cerevisiae*
8.000 [8]	10	12	94,716	96,990	*Saccharomyces cerevisiae*
8.000 [12]	15	9	71,037	92,244	*Pichia Stipitis*

In table 1 is shown that the highest of bioethanol yield produced from fermentation of nypa sap is done in [11], that is equal to 97.969%. This shows the performance of fermentaion of nypa sap using *Saccaromyces cerevisiae* yeast were better than the fermentation of nypa sap using *Pichia stipitis* yeast under which produces bioethanol yield gains in [11].

The high concentrations of ethanol are also influenced by the volume of starter used. The highest concentration of bioethanol produced in volume 10% starter. The increasing volume of starter it will reduce levels of ethanol fermentation, it is because in addition to the metabolic processes that convert substrates into products, microorganisms also require most substrates for growth, both in reproduction form new cells as well as increasing the size of the cell, so that not all substrate is converted into product.

In addition, the volume of substrate that used also affects the concentration of bioethanol produced. In the study [11], the resulting bioethanol concentration is higher than other studies in the amount of 14% by volume of 50 liters of substrate. This, due to the availability of nutrients for microorganisms metabolic processes so as to produce more higher.

IV. CONCLUSION

The sugar content in the nypa sap is about 15-17%. Consequently the nypa sap is sufficient to be used as alcohol fermentation feedstock. In alcohol production using nypa sap as the feedstock, the best consentration and yields was obtained when the fermentation was treated with 10% of starter, 50L of substrat volume, and using Saccaromyces cerevisiae. The bioethanol consentration and yield were obtained by 14% (v/v) and 97.969% of yield.

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